RESEARCH REPORT
SWINE HEALTH

pork checkoff

Title: Evaluation of Mortality Composting Parameters Necessary for Inactivation of PEDv – **NPB #14-239**

Investigator: Amy Millmier Schmidt

Institution: University of Nebraska, Lincoln, NE

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Industry Summary

This project was conducted in an effort to determine the transmission risk for PEDv following on-farm composting of PEDv-positive swine mortalities. The studies performed were designed to quantify the persistence of PEDV RNA via quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) at various time-temperature combinations and in infected piglet carcasses subjected to composting.

Specific objectives of this project were to:

1.) Identify appropriate time-temperature combinations for inactivation of PEDv in compost material.
2.) Validate time-temperature combinations for PEDv inactivation in mortality compost piles.
3.) Increase confidence among pork producers and their advisors in utilizing on-farm composting as a biosecure method for disposing of PEDv-infected animals.

Identification of Time-Temperature Combinations for PEDv Inactivation (Obj. 1)
This study was conducted to quantify the persistence of PEDv RNA via quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) at various time-temperature combinations under controlled laboratory conditions.

Porcine Epidemic Diarrhea Virus was inoculated into cell culture media at 1 x 10^5 TCID50 per sample (1 mL sample size) at various time and temperature combinations including temperatures of 37, 45, 50, 55, 60, 65, 70°C and exposure times of 0, 24, 48, 72, 96, 120, 168 and 336 hrs. The temperature treatments were performed in incubators (Heratherm General Protocol Incubator, Thermo Fisher Scientific, Waltham, MA) maintained at the target temperatures, which were monitored for consistency throughout the trial. At each designated time point in the experiment, three cryovials were removed from the incubator and transferred to -80°C for storage until analysis by qRT-PCR. At all temperatures, virus RNA copies declined over time and with the decline most marked and rapid for temperatures of 65 and 70°C. Two of three samples had undetectable virus RNA after 336 hrs incubation at 70 °C.

These research results were submitted in fulfillment of checkoff-funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer-reviewed.

For more information contact:
National Pork Board • PO Box 9114 • Des Moines, IA 50306 USA • 800-456-7675 • Fax: 515-223-2646 • pork.org

Impact of Composting on Inactivation of PEDv (Obj. 2)

This study was conducted to quantify the persistence of PEDv RNA via quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) following composting of PEDV-positive piglets.

Three insulated platforms were constructed on wood pallets with internal bin dimensions of 121.92 cm (W) x 93.46 (L) cm x 97.53 cm (H). Platform walls were constructed of an outer layer of plywood (12.7 mm) and an inner layer of non-porous PolyBoard sheeting (4 mm) with foam board insulation (24 mm) placed between these layers to achieve a composite R-value of 17.3 m^2KW^{-1}. This effort was taken to simulate the linear continuation of the windrow and the insulative properties of a compacted soil base.

A single trial was conducted with a compost windrow constructed in each of the three bins using pine wood shavings at a mean moisture content of 55% w.b. Five 21-day-old piglets experimentally infected with PEDv were composted in each of the three bins over two compost cycles lasting 31 and 27 d, respectively, and temperature was monitored throughout piles during each cycle. Compost samples were collected at ten locations in each pile at the completion of the first and second compost cycles and analyzed for PEDv RNA using RT-qPCR. Material samples taken from all locations within the three piles had undetectable viral RNA following each compost cycle.

Producer Outreach (Obj. 3)

Results and recommendations regarding composting as a biosecure mortality disposal method for PEDV-infected pig carcasses have been disseminated via several routes. A UNL NebGuide (*G2280: Composting of PEDv-Positive Swine Mortalities*) has been published and is available online at http://extensionpublications.unl.edu/assets/html/g2280/build/g2280.htm. Results and recommendations have been incorporated into the December 2015 National Pork Board publication *PEDV Resources: PEDV Brings Its Worst. Pork Checkoff Brings Its Best*, available online at http://www.pork.org/wp-content/uploads/2013/11/pedvbookjan16final.pdf.

Popular press articles and related media were distributed as follows:

Recent progresses in UNL swine disease research and disease update: Porcine Epidemic Diarrhea, Nebraska Pork Talk Magazine, January/February 2016, p. 5.

UNL Undergraduate Students Active on Swine-Related Research: Bethany Brittenham, Nebraska Pork Talk Magazine, November/December 2015, p. 8.

On-farm Remediation and Prevention of Swine Enteric Diseases, Nebraska Pork Talk Magazine, May/June 2016, p. 20.

UNL PEDv Research, KTIC Radio Station Podcast, May 2016:
http://ktic.podbean.com/e/unl-pedv-research/

Composting PEDV-infected Carcasses May Help Its Control, PorkNetwork.com, April 2016:
http://www.porknetwork.com/pedv/composting-pedv-infected-carcasses-may-help-its-control.

Improved Methods to Control Porcine Epidemic Diarrhea Virus, Veterinary Medicine Blog, April 2016:
https://veterinarymedicineechbeebolanle-ojuri.blogspot.com/2016/04/improved-methods-to-control-porcine.html

UNL Research Aimed at Improving Swine Disease Control, IANR News, February 2016:
http://ianrnews.unl.edu/unl-research-aimed-improving-swine-disease-control

Learning from PEDv: Managing disease in manure and mortalities, iGrow.org, November 2015: http://igrow.org/livestock/pork/learning-from-pedv-managing-disease-in-manure-and-mortalities/

PEDV Survivability in Swine Mortality Compost Piles, eXtension.org, March 2015: http://articles.extension.org/pages/72851/pedv-survivability-in-swine-mortality-compost-piles

Managing Biosecurity to Control Porcine Epidemic Diarrhea virus (PEDv), eXtension.org, July 2014: http://articles.extension.org/pages/71083/managing-biosecurity-to-control-porcine-epidemic-diarrhea-virus-pedv

Conclusions garnered from this project include:

1. Composting of PEDV-infected piglets is an effective and biosecure method of mortality disposal.
2. The combination of time and high temperature of the compost cycle has been shown to effectively degrade virus RNA in matrices that promote stability, such as cell culture media.
3. Complex compost material matrices from demonstration piles had undetectable PEDV RNA by qRT-PCR after one 35-d compost cycle.
4. Exposure temperature and duration of exposure appear to have less impact on virus survival than pH of the media in which the virus is contained.
5. In aqueous solutions (i.e. water, manure, etc.), pH has a significant impact on virus survival with higher pH being more detrimental to the virus. Additional work is being conducted to delineate the relationship between pH and virus inactivation.

Contact Information: Dr. Amy Millmier Schmidt, University of Nebraska – Lincoln, aschmidt@unl.edu, (402) 472-0877

Keywords: *PEDV, mortality disposal, composting, biosecurity*

Scientific Abstract

A large body of research on virus persistence has been conducted to assess viability of the virus on vehicles, in feed and water, and on production building surface, little is known about the persistence in and effective disposal methods for PEDV-infected carcasses. This project was conducted to quantify the persistence of PEDV RNA via quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) at various time-temperature combinations and in infected piglet carcasses subjected to composting. Porcine Epidemic Diarrhea Virus was inoculated into cell culture media at 1×10^5 TCID50 per sample (1 mL sample size) at various time and temperature combinations including temperatures of 37, 45, 50, 55, 60, 65, 70 °C and exposure times of 0, 24, 48, 72, 96, 120, 168 and 336 hrs. At all temperatures, virus RNA copies declined over time and was most marked and rapid for 65 and 70 °C. Two of three samples had undetectable virus RNA after 336 hrs incubation at 70 °C. PEDV-infected piglet carcasses were subjected to two cycles of composting lasting 35 d and 30 d, respectively, for a total compost time of 65 d. The compost process was replicated in three windrow sections contained within constructed bins. Bins were designed with insulated walls to represent a continuous windrow compost pile. Material samples taken from ten locations within the pile representative of the pile cross-section had undetectable viral RNA following each cycle.

Introduction

The large number of carcasses and volume of infected material generated during PEDV outbreaks has been problematic, and proven, biosecure methods for mortality disposal are sought to control the outbreak on-farm and limit site-to-site transmission. Major mortality disposal methods available to swine producers include rendering, incineration, burial, land-filling and composting. Composting is an attractive option because it requires relatively low input costs, poses little environmental risk when properly designed and managed, and

offers greater biosecurity than methods involving transport of infected carcasses beyond the farm boundary. Furthermore, in situations of disease-associated mortalities, composting is capable of inactivating the pathogen of concern when the system is managed to achieve and maintain pile temperature targets.

PEDV is an enveloped virus demonstrated to be sensitive to a variety of disinfectants, extremes of pH, and elevated temperature. Therefore, composting is likely to be an effective method of virus elimination. The present study was performed to evaluate the persistence of PEDV RNA with qRT-PCR in matrices and temperature conditions mimicking field conditions of composting in order to determine the potential effectiveness of this method for PEDV mortality disposal. The study consisted of a laboratory phase examining the rate of virus RNA loss in a controlled application of time and temperature combinations in virus media, and a composting phase where PEDV-infected piglet carcasses were incorporated into compost bins and periodically tested.

Objectives

This project was conducted to achieve the following research and outreach objectives:

1.) Identify appropriate time-temperature combinations for inactivation of PEDv in compost material.
2.) Validate time-temperature combinations for PEDv inactivation in mortality compost piles.
3.) Increase confidence among pork producers and their advisors in utilizing on-farm composting as a biosecure method for disposing of PEDv-infected animals.

Materials & Methods

Identification of Time-Temperature Combinations for PEDv Inactivation (Obj. 1)

Vero cells were maintained in minimal essential media (MEM) containing 10% fetal bovine serum and 100 µg/mL gentamicin. Two day old confluent monolayers of Vero cells in 150 cm^2 flasks were washed 2 times with MEM containing 2 µg/mL L-(tosylamido-2-phenyl) ethyl chloromethyl ketone (TPCK)-treated trypsin prior to inoculation. Monolayers were infected at approximately 0.01 multiplicity of infection (MOI) of PEDV in MEM containing 2 µg/mL TPCK-treated trypsin, and incubated at 37ºC until maximum cytopathic effect (CPE) (48 to 96 h). Flasks were cycled through two brief freeze-thaw cycles, and stored at -80ºC until further processing. For purification, frozen flasks were thawed and the contents centrifuged at 3000 rpm for 10 min. The media supernatant was collected, pooled, mixed, and divided into aliquots which were then stored at -80ºC until needed.

The trial was constructed with combinations of 37, 45, 50, 55, 60, 65, 70°C and exposure times of 0, 24, 48, 72, 96, 120, 168 and 336 hrs (0, 1, 2, 3, 4, 5, 7, and 14 days), with three virus samples per time-temperature treatment. Each virus sample was allocated into a sealed cryovial at a volume of 1 mL and was comprised of 1 x 10^5 TCID50 of cell-culture propagated PEDV in MEM containing 2 µg/mL TPCK-treated trypsin, 10% fetal bovine serum, and 100 µg/mL gentamicin. The temperature treatment was performed in an incubator (Heratherm General Protocol Incubator, Thermo Fisher Scientific, Waltham, MA), which was monitored for temperature consistency throughout the trial. Following treatment, the cryovial was transferred to -80°C for storage until qRT-PCR.

The qRT-PCR sample was diluted 1:2 prior to testing to conserve sample. Each RT-PCR sample was run on a Cepheid Smart Cycler Detection System. Validated PCR positive controls consisting of PEDV RNA and negative extraction controls were included in each run. Samples were considered positive if the mean fluorescence exceeded 30 fluorescent units prior to 40 cycles and negative and positive PCR controls were properly classified.

Impact of Composting on Inactivation of PEDv (Obj. 2)

Twenty-seven 21-day-old piglets were acquired from a PED-naïve herd and confirmed negative for PEDv by RT-PCR via rectal swab on arrival to the facility. The sows from the herd of origin were serologically negative by PEDv indirect immunofluorescence assay (IFA). The piglets were allowed 3 days of acclimation prior to the start of the study and maintained on a commercial diet free of plasma products. Virus stock and MEM were mixed so that each piglet inoculum was a 5 mL volume containing 1 X 10^6 TCID50 of PEDv. This inoculum was administered to piglets via syringe and gavage needle immediately following dilution.

Fecal swabs were collected at days 3 and 5 post-infection to confirm infection via viral shedding. Humane euthanasia and necropsy were performed on day 5 post infection. Fresh and formalin fixed tissues were collected, including two segments of jejunum, one segment of ileum, and mesenteric lymph node. The remaining carcass was incorporated into the compost bin housed within the biosecure room.

Insulated platforms (Figure 1) were constructed on wood pallets measuring 121.92 cm (W) x 101.6 cm (L). Internal dimensions of platforms were 121.92 cm (W) x 93.46 (L) cm x 97.53 cm (H) to contain a windrow section. Platform walls were constructed of an outer layer of plywood (12.7 mm) and an inner layer of non-porous PolyBoard sheeting (4 mm). Foam board insulation (24 mm) was placed between these layers to achieve a composite R-value of 17.3 m^2KW^{-1}. This effort was taken to simulate the linear continuation of the windrow and the insulative properties of a compacted soil base.

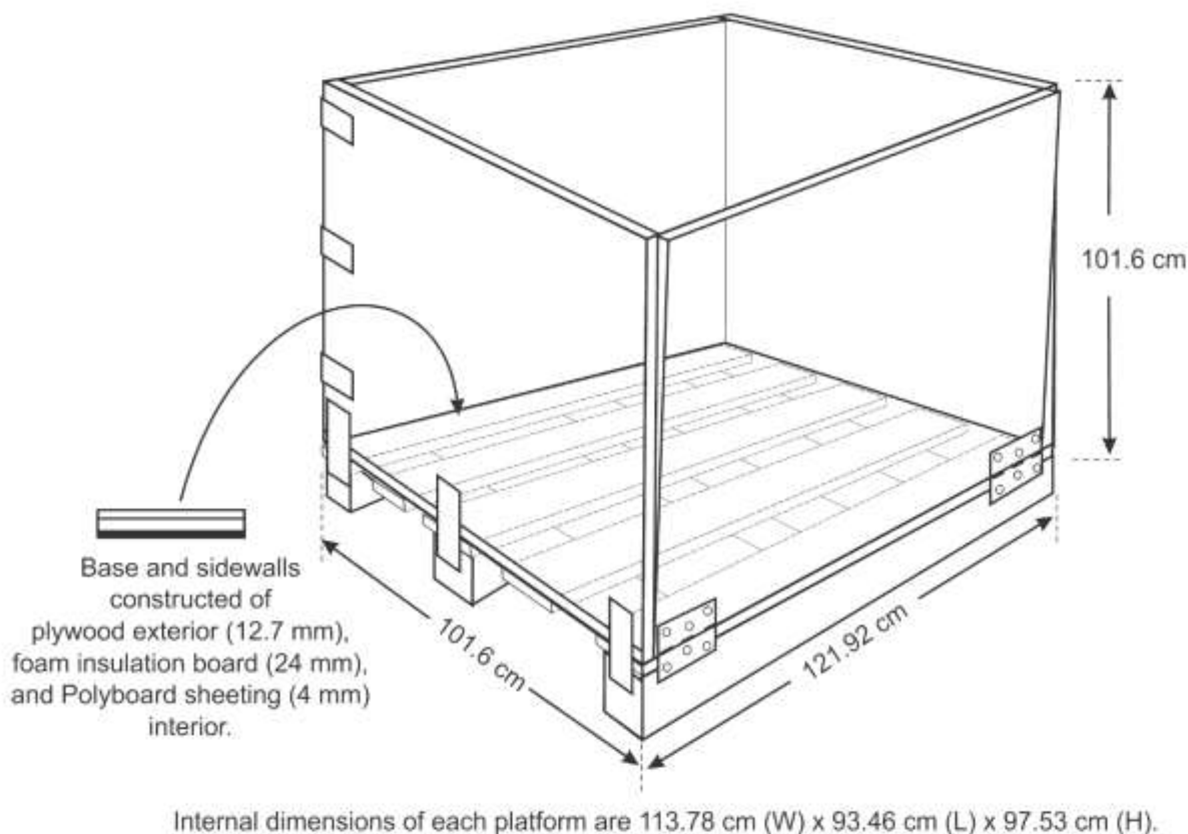

Figure 1. Windrow compost bin design

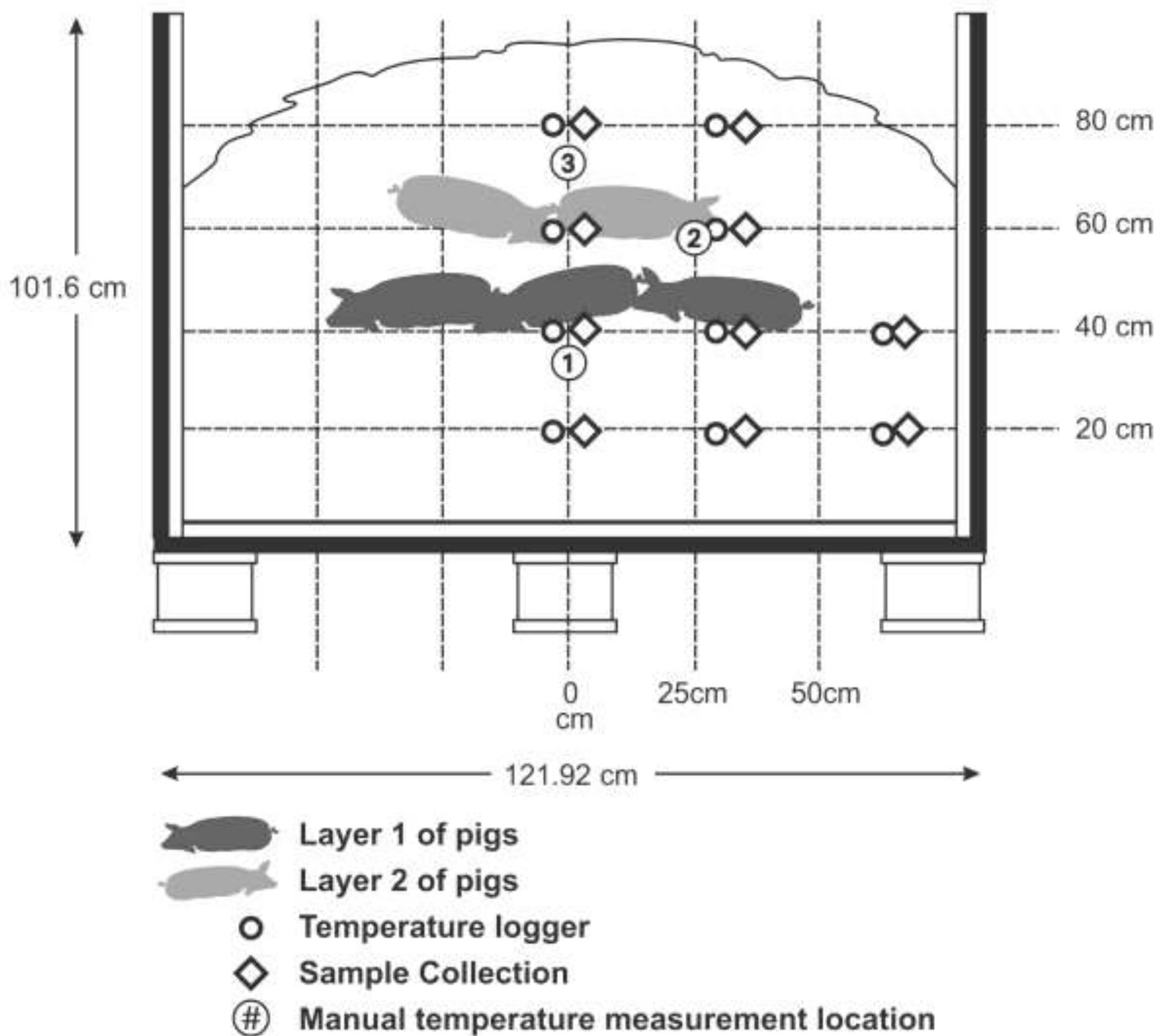

Figure 2. Windrow compost pile construction, temperature monitoring locations, and sample collection points

Windrow test sections were constructed within the platforms (Figure 2) and a single trial was conducted with three windrow test sections. Room conditions were maintained at 16-27°C and 50-80% relative humidity for the duration of the trial. Pine wood shavings wetted to a target moisture content of 55-60% w.b. were used to construct the windrows in layers to allow placement of temperature recorders. At the midpoint of each pile, three animals were placed along the centerline of the pile with minimal space between animals. A 10-cm layer of wood shavings was placed across the entire pile and then two additional animals were placed along the centerline of the pile. At least 30 cm of clearance was maintained between the animals and platform walls for both layers of animals.

Compost temperature in the windrows was measured on a 25-cm x 20-cm spatial grid with Apresys Temp Trak Temperature Recorders (Figure 2). Symmetry was assumed along the vertical centerline of the windrow section to minimize the number of spatial sampling locations. Temperatures were recorded on a temporal sampling interval of 20 min throughout each compost cycle. Manual temperature measurements were also collected throughout each compost cycle at three locations in each windrow (Figure 2) using a long-handled compost

thermometer (REOTEMP Instrument Corp., San Diego, CA). The thermometer was inserted and allowed to stabilize for at least 1 min prior to temperatures being recorded.

Two consecutive compost cycles, lasting 31 and 27 d, respectively, were performed. Manual temperature monitoring was conducted regularly (daily during the first week of each compost cycle and at least every three days beyond the first week of each cycle) to monitor progression of the compost process. When temperatures failed to increase or declined, water was added to piles from a municipal source to achieve a damp sponge feel of the carbon material. In response to a slow temperature rise in all piles, 5.5 kg of Roebic compost accelerator was added to each pile on day 2 of the first compost cycle. On day 5, 0.4 kg of granular fertilizer (Lesco 18-0-18 fertilizer, Home Depot, Lincoln, NE) was added to all windrows to further accelerate microbial activity. To add both the compost accelerator and granular fertilizer, carcasses were carefully uncovered and the products were evenly distributed across the exposed pile surface. Carbon material was then returned to the original configuration following fertilizer addition.

Once manual temperature measurements reflected a sustained temperature of less than 100°C in all windrows, windrows were disassembled, temperature recorders were retrieved, compost material samples were collected, and piles were reconstructed following the same protocol described previously to initiate a second compost cycle. During the second compost cycle, temperatures were monitored as previously described and moisture added as needed. As with the first compost cycle, once temperature measurements reflected a sustained temperature of less than 100°C in all windrows, windrows were disassembled for retrieval of temperature recorders and collection of compost material samples and remaining materials were incinerated.

Compost samples were collected at ten locations in each pile (Figure 2) corresponding to temperature recorder locations at the completion of the first and second compost cycles. To collect samples, piles were carefully deconstructed in layers until temperature recorders were exposed. Grab samples of approximately 50 g of compost material were then collected near the location of each temperature recorder and placed into sterile Whirl-pak bags (Nasco, Fort Atkinson, WI). A new pair of nitrile exam gloves was worn during each individual sample collection. Samples were stored at -80ºC until testing.

For processing, 30 g of sample was placed into a sterile Whirl-pak bag along with 50 mL of MEM containing 100 µg/mL gentamicin. The bag was closed and subjected to vigorous manual blending for 2 min, assuring that all compost material and organic matter/bones had been thoroughly washed with media solution. The excess media was separated into a 50 mL conical vial and stored at -80ºC until testing.

For processing, 20 g of sample was placed into a sterile Whirl-pak bag along with 50 mL of MEM containing 100 µg/mL gentamicin. The bag was closed and subjected to stomacher blending for 2 min at 230 RPM, assuring that all compost material and organic matter had been thoroughly washed with media solution. The supernatant media was separated into a 50 mL conical vial and stored at -80ºC until qRT-PCR.

The qRT-PCR sample was diluted 1:2 prior to testing to conserve sample. Each RT-PCR sample was run on a Cepheid Smart Cycler Detection System. Validated PCR positive controls consisting of PEDV RNA and negative extraction controls were included in each run. Samples were considered positive if the mean fluorescence exceeded 30 fluorescent units prior to 40 cycles and negative and positive PCR controls were properly classified.

Results

Objective 1. Identify appropriate time-temperature combinations for inactivation of PEDv in compost material.

At all temperatures, virus RNA copies declined over time and was most marked and rapid for 65 and 70 °C. Two of three samples had undetectable virus RNA after 336 hrs incubation at 70 °C.

Objective 2. Validate time-temperature combinations for PEDv inactivation in mortality compost piles.

Material samples taken from ten locations within the pile representative of the pile cross-section had undetectable viral RNA following each cycle.

Objective 3. Increase confidence among pork producers and their advisors in utilizing on-farm composting as a biosecure method for disposing of PEDv-infected animals.

Multiple popular press articles, media interviews, and extension publications have been distributed to producers and stakeholders. A survey of knowledge and behavior changes is being developed to quantify impacts of the outreach efforts. Examples of feedback received regarding the work conducted include the following:

"I just want to…extend my appreciation for what you…are doing…to find out more about PED through your research. This…[information]…could be very beneficial to our industry as it will help us learn more about PED so we can more effectively prevent and or eliminate the transmission of this costly disease." – Nebraska Pork Producer

"It has been a pleasure to…cooperate with [your research team] on studies to…identify manure and mortality [management methods]…to [control] PEDV. [Knowing how to]…handle manure and [mortalities] following PEDV is important and your research has helped [us] feel confident that we are doing the right things to keep from spreading this devastating disease. Thank you!" – Nebraska Pork Producer

Discussion

Important conclusions and recommendations resulting from this project are:

1. Composting appears to be an effective disposal method for PEDV-infected piglet carcasses under the conditions examined.
2. The combination of time and high temperature of the compost cycle has been shown to effectively degrade virus RNA in matrices that promote stability, such as cell culture media.
3. Complex compost material matrices from demonstration piles had undetectable PEDV RNA by qRT-PCR after one 35-d compost cycle.
4. Exposure temperature and duration of exposure appear to have less impact on virus survival than pH of the media in which the virus is contained.
5. In aqueous solutions (i.e. water, manure, etc.), pH has a significant impact on virus survival with higher pH being more detrimental to the virus. Additional work is being conducted to delineate the relationship between pH and virus inactivation.

When composting mortalities, it is important to utilize sufficient carbon material to insulate the pile and promote heating, maintain a "wet sponge" feel to the carbon material to promote microbial activity that drives the compost process, and keep the pile covered with at least 24" of dry carbon material to prevent odors and retain heat necessary to inactivate pathogens. While the study presented demonstrated that a single 35-d compost cycle was effective for eliminating PEDV RNA, additional composting is typically required to break down soft tissue and yield a product suitable for land application.